

What is claimed is:

1. A method for detecting and quantifying telomerase activity in a biological sample, the method comprising the steps of:

adding the biological sample to a reaction tube comprising:

5 a first reaction mixture comprising a first primer and nucleoside triphosphates;

a second reaction mixture comprising a second primer and a DNA polymerase; and

10 a wax layer separating the first reaction mixture from the second reaction mixture in the reaction tube;

incubating the biological sample with the first reaction mixture under conditions suitable for a telomerase to produce an extension product from the first primer, said extension product having a 3' end;

15 admixing the extension product with the second reaction mixture by melting the wax layer;

amplifying the extension product using a real-time polymerase chain reaction under conditions that allow the detection of telomerase activity from a single 293T cell; and

quantifying the amplified extension product using a control template.

20 2. The method of claim 1, wherein the biological sample is added in the form of a cell or tissue extract.

3. The method of claim 1, wherein the real-time polymerase chain reaction is quantified by using a fluorescently labeled probe oligonucleotide that binds to a sequence between the first and the second primers.

25 4. The method of claim 1, wherein the real-time polymerase chain reaction is performed in the presence of a fluorescent dye that binds preferentially to double-stranded DNA.

5. The method of claim 1, wherein the second primer is a single-labeled fluorogenic primer that produces an increased amount of fluorescence emission when the fluorogenic primer is incorporated into double-stranded polymerase chain reaction product.

30 6. The method of claim 1, further comprising:

elongating the extended product at the 3' end by one of polyadenylation and ligation.

7. The method of claim 1, wherein the control template has a nucleotide sequence recited in SEQ ID NO:2.

8. A method for detecting and quantifying telomerase activity in a sample cell, the method comprising the steps of:

5       introducing into a sample cell a first primer and nucleoside triphosphates;  
      incubating the sample cell under conditions suitable for a telomerase to produce an extension product from the first primer;

      amplifying the extension product using real-time polymerase chain reaction; and  
      quantifying the amplified extension product using a control template.

10   9. The method of claim 8, further comprising:  
      lysing the sample cell with a lysis buffer.

10. The method of claim 8, wherein the first primer and nucleoside triphosphates are introduced into the sample cell by calcium phosphate precipitation.

15   11. The method of claim 8, wherein the first primer and nucleoside triphosphates are introduced into the sample cell by a procedure comprising:

      passing the cell through a needle at least once; and

      culture the cell in a culture medium containing the first primer and nucleoside triphosphates.

20   12. The method of claim 8, wherein the real-time polymerase chain reaction is performed in the presence of a fluorescent dye that binds preferentially to double-stranded DNA.

25   13. The method of claim 8, wherein the real-time polymerase chain reaction is performed in the presence of a second primer, and wherein the second primer is a fluorogenic primer that produces an increased amount of fluorescence emission when the fluorogenic primer is incorporated into double-stranded polymerase chain reaction product.

14. The method of claim 8, wherein the control template has a nucleotide sequence recited in SEQ ID NO:2.

30   15. A method for detecting and quantifying telomerase activity in a biological sample, the method comprising the steps of:

      adding the biological sample to a reaction tube comprising:

          a first reaction mixture comprising a first primer and nucleoside triphosphates;

a second reaction mixture comprising a second primer and a DNA polymerase; and

a wax layer separating the first reaction mixture from the second reaction mixture in the reaction tube;

5 incubating the biological sample with the first reaction mixture under conditions suitable for a telomerase to produce an extension product from the first primer, said extension product;

elongating the extended product at a 3' end by one of polyadenylation and ligation;

10 admixing the extension product with the second reaction mixture by melting the wax layer;

amplifying the extension product using a real-time polymerase chain reaction under conditions that allow the detection of telomerase activity from a single 293T cell; and

15 quantifying the amplified extension product using a control template, wherein the second primer comprises a nucleotide sequence that is complementary to the nucleotide sequence at a 3' end of the elongated extension product.

16. A kit for detecting telomerase activity, the kit comprising:  
reaction tubes comprising:

20 a first reaction mixture comprising a first primer and nucleoside triphosphates;

a second reaction mixture comprising a second primer and a DNA polymerase; and

25 a wax layer separating the first reaction mixture from the second reaction mixture in the reaction tube, and

control tubes comprising:

the first reaction mixture;

a third reaction mixture comprising the second primer, a control template, and the DNA polymerase; and

30 a wax layer separating the first reaction mixture from the third reaction mixture in the control tube.

17. The kit of claim 16, wherein the control template has a nucleotide sequence recited in SEQ ID NO:2.

18. The kit of claim 16, further comprising:

an elongation mixture comprising (a) a DNA polymerase or (b) a ligase and an oligonucleotide.

19. A kit for detecting telomerase activity, the kit comprising:

a lysis buffer for lysing sample cells;

5 a reaction buffer comprising:

a first primer;

nucleoside triphosphates;

a second primer; and

a DNA polymerase; and

10 a control template comprising the nucleotide sequence recited in SEQ ID NO:2 or a nucleotide sequence complementary to the nucleotide sequence recited in SEQ ID NO:2.

20. A method for monitoring the effectiveness of treatment of a subject with an agent that inhibits telomerase activity, said method comprising:

15 obtaining a pre-administration sample from the subject prior to administration of the agent;

detecting a level of telomerase activity in the pre-administration sample;

obtaining one or more post-administration samples from the subject;

detecting the level of telomerase activity in the post-administration samples; and

20 comparing the level of telomerase activity in the pre-administration sample with the level of telomerase activity in the post-administration sample or samples.